THE LIPIDS OF GERMINATING WHEAT

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ABSTRACT.

The evolution of the fatty acids present in the lipid classes of different structural parts of the wheat kernel during germination in the dark has been investigated.

Lipid was extracted with chloroform: methanol (2:1), washed according to Folch *et al.*, and fractionated by preparative TLC. Free fatty acids (FFA), as well as those present in triglycerides (TGFA), diglycerides (DGFA), monoglycerides (MGFA), polar lipids (PLFA), and the total lipid extract (TFA) have been determinated by GLC.

In all parts studied, the PLFA pattern is rather constant and, the rootlets excepted, so is their content in the tissues (% of dry matter). Observed changes in lipid content and composition are mainly due to the glyceride fractions.

In the degermed seed, lipid is lost at a faster relative rate than non-fat dry matter. The TGFA pattern shows a constant proportion of palmitic ($C_{16:0}$), stearic ($C_{18:0}$), and linolenic ($C_{81:3}$), but that of linoleic ($C_{18:2}$), increases at the expense of oleic ($C_{18:1}$), as germination proceeds.

A net loss of lipid followed by a net synthesis is observed in the germ during germination. A drastic change in TGFA pattern takes place during the first half of the process. The data suggests that the original triglycerides present in the germ are catabolized very rapidly while new triglycerides with a different fatty acid pattern are synthesized in the growing tissues.

INTRODUCTION

Lipid metabolism of higher plants has been investigated most actively in recent years (1,2). Extensive data has accumulated on the changes of lipid composition during germination in oil-bearing seeds (3-9 & others). Considerably less information is available concerning lipid changes in the germination of starchy seeds. Recent work with cereals has been restricted to changes in the free and sterified fatty acids as extracted by petroleum ether from barley (10). The present study deals with the evolution of the fatty acids present in the lipid clases of different structural parts of the wheat kernel during germination. It has been undertaken in connection with previous work from this laboratory on wheat lipases (11), as well as being a part of a more general study on the biochemical and nutritional properties of germinated wheat.

chloroform: ethanol (2:1) for iii) and iv); an chloroform: methanol: water (2:1:0.2) for v) and homologous fractions in separation B. The eluates were collected in glasse tubes, 2 cm. in diameter.

Quantitative analysis of fatty acids. — Determination of fatty acids present in the above fractions was carried out by GLC of their methyl esters. A methyllating reagent based in that of Glass et al. (14) was used: two internal standards, methyl undecanoate (170 mgr.) and methyl tridecanoate (17 mgr.) were brought up to 100 ml. with a mixture of dimethyl carbonate-methanol-benzene (3:2:5). An exact volume of this reagent (0.5-2.0 ml.) was added to each fraction, the elution solvent being eliminated previously by adapting the ground glass tubes to a flash evaporator. The solution was made alkaline with 2N sodium methoxide, using phenolftalein as an indicator. After 20 minutes, 2N methanolic hydrochloric acid was added until acid pH and the mixture allowed to stand for another 45 minutes before chromatography.

Gas liquid Chromatography was carried out in a F & M Model 609 Gas Chromatograph with a flame ionization detector. The 5 feet alluminum colum was filled with diethylenglycol succinate over Diaport W (both F & M Scientific Corporation).

The sample (10-50 microliters) was injected at 110° C and, after 2 minutes, a 13° C./minute temperature increase was programmed to reach a maximum of 200° C. Identification of the methyl esters was achieved by comparison of their retention times with those of authentic standards (Hormel Institute) and by co-chromatography.

Quatitative determination was made by comparison of peak area of unknown with that of the internal standards. The tenfold difference in concentration of the later and the different amounts of methylating reagent added to each sample allowed the analysis of a wide range of concentration. Only the major fatty acids of wheat, palmitic (C 16:0), stearic (C 18:0), oleic (C 18:1), linoleic (C 18:2) and linolenic acid (C 18:3) were studied. For the fatty acids present in the sterol esters fraction only a maximum value was obtained because due to other components also eluted with that fraction, there was considerable overlapping with unidentified peaks. These values were well under 1 % of the total fatty acids in all samples and have not been reported.

Recovery of the preparative TLC procedure was obtained by comparing the quantitative GLC analysis of the unfractionated lipid samples with the sum of the fractions. This was estimated to be 94-96 %.

RESULTS AND DISCUSSION

Dry weight changes. — Dry weifht changes in our experimental conditions are presented in table 1. Total dry weight loss was 9.6 % for the 9 days period, the 21,9 % loss due to endosperm degradation being partly compensated by the rapid growth of the germinating axis. Seed coat weight remained practically constant throughout.

TABLE 1. — *Dry weight changes of structural parts of wheat Kernel in mg./10 seeds.*

Germination stage	Roots	Rest of germ	Seed coats	Endosperm	TOTAL
Dry seed	—	—	—	—	251
Steeped (*)	—	3	51	196	250
36 hr.	1	5	56	183	245
72 hr.	6	6	49	183	244
108 hr.	8	9	55	171	243
144 hr.	10	13	54	164	241
180 hr.	12	14	55	153	234
218 hr.	13	15	58	141	227

(*) No separation of the rootlets was attempted in this sample.

Changes in lipid extract and fatty acid content. — A decrease in the chloroform : methanol (2 : 1) extract and total fatty acid content (both as % of dry matter) of both the germ and the rest of the seed is observed during germination (fig. 1).

For the endosperm, which is being degraded, it means that the relative rate of lipid utilization is greater than that of non-fat dry matter. The opposite behaviour has been reported by Brown et al. (7) for the first 2 days of germination of soybean. The faster rate of fat utilization is due to the preferential degradation of triglyceride fatty acids (TGFA), the content (% of dry matter) of polar lipid fatty acids (PLFA) remaining constant and that of the free and partial glyceride fatty acids (FFA, DGFA and MGFA) slightly increasing during the period of maximum metabolic activity (fig. 1, *c*).

Lipid content (% of dry matter) of the germ (fig. 1, *a* and 1, *b*) decreases sharply during the first half of the germination period and

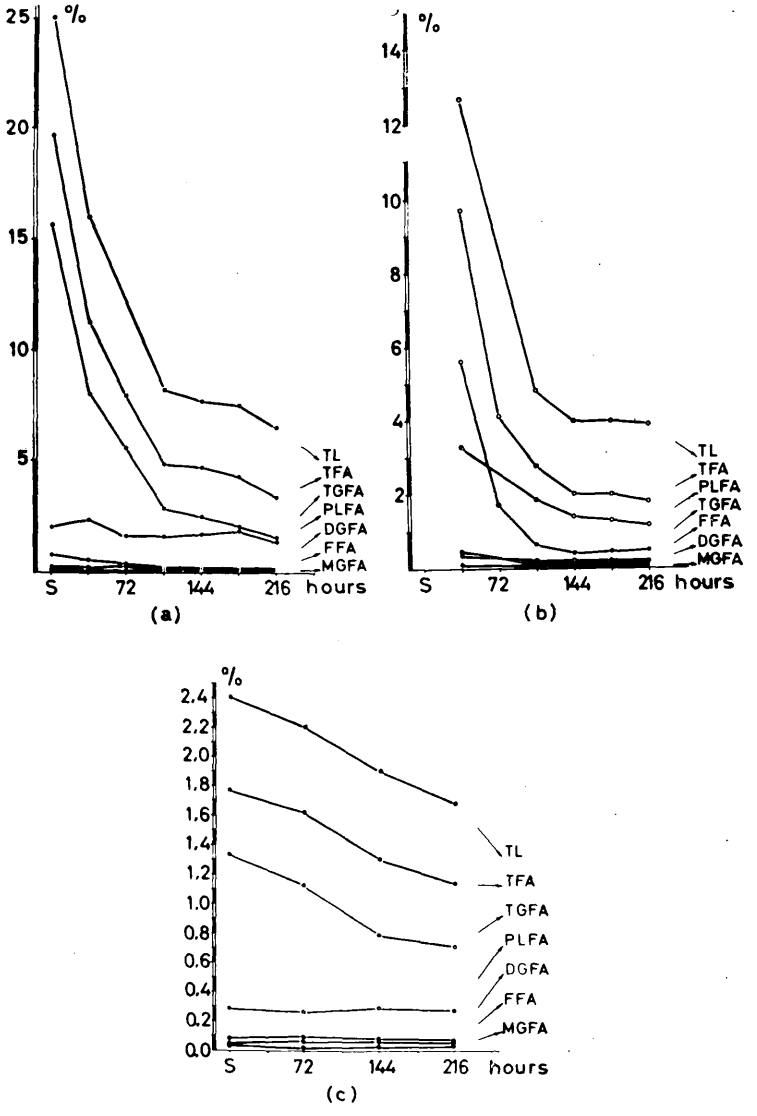

Figure 1.—Changes in lipid extract and fatty acids content (% of dry matter) during germination. Germ minus rootlets (*a*); Rootlets (*b*); Degermed seed (*c*). Lipid extract (TL) according to Folch et al. (12); Fatty acids present in total extract (TFA); in Triglycerides (TGFA) in Diglycerides (DGFA); in Monoglycerides (MGFA); in Polar Lipids (PLFA). Steeped (S) germ was not deprived of rootlets.

slowly during the second. Lipid degradation is faster than dry weight increase at first and slower afterwards, and so a net loss of lipid occurs in the first stages followed by a net synthesis. This does not seem to be the case for some oil bearing seeds where a constant total fat per seedling axis has been reported (4, 7). As in the endosperm, TGFA account for most of the lipid content decrease. As the seedling develops, PLFA level in the rootlets declines somewhat but this fraction soon becomes dominant due to the sharp disappearance of TGFA (fig. 1, *b*). In the rest of the germ PLFA content remains constant (fig. 1, *a*). The levels of MGFA, DGFA and FFA are slightly higher during the first three days of germination when most of the germ TGFA degradation takes place.

It might be intersting to note that while FFA level is intermediate berween DGFA and MGFA both in the endosperm and in the "rest of the germ", in the rootlets it becomes higher than either. This could be relatel to the fact that polar lipids metabolism is dominant in this part of the germ but no acceptable explanation can be offered.

The decreasing fatty acid content (TFA) of the lipid extracts (TL) of all seed parts studied can be explained, at least in part, by the increasing proportion of polar lipids in them, an increase in the insaponibiable not being excluded.

Changes of fatty acids pattern in lipid classes during germination. The overall fatty acid pattern (TFA) of the degermed seed (fig. 2) shows an increasing proportion of linoleic acid (C 18 : 2) and a decreasing one of oleic (C 18 : 1), while that of palmitic (C 16 : 0), stearic (C 18 : 0) and linolenic (C 18 : 3) do not change. The PLFA pattern in practically constant, so the TGFA are mainly responsible of the above changes. The same trend can be observed in FFA and to a lesser degree in MGFA. It is to be noted that FFA, DGFA and MGFA show a greater proportion of saturated fatty acids than either TGFA or PLFA. The analysis of two partially pure endosperm samples (steeped and 218 hr. germinated) gave superimposable patterns and trends for both TGFA but a still greater proportion of saturated fatty acids in FFA, MGFA and MGFA than that observed in the degermed samples (endosperm plus coats).

As in wheat, a faster depletion of oleic acid has been reported in barley by McLeod and White (10) and in peanut seeds by Rabari et al. (5). However, this does not seem to be the general case, for a non-selective metabolism of fatty acids has been reported in several germi-

nating seeds by Crombie and Comber (4) and a preferential utilization of linoleic acid has been shown in Douglas fir seed (9) and cotton seed (8), while Brown et al. (7) have found that oleic acid degradation was slo-

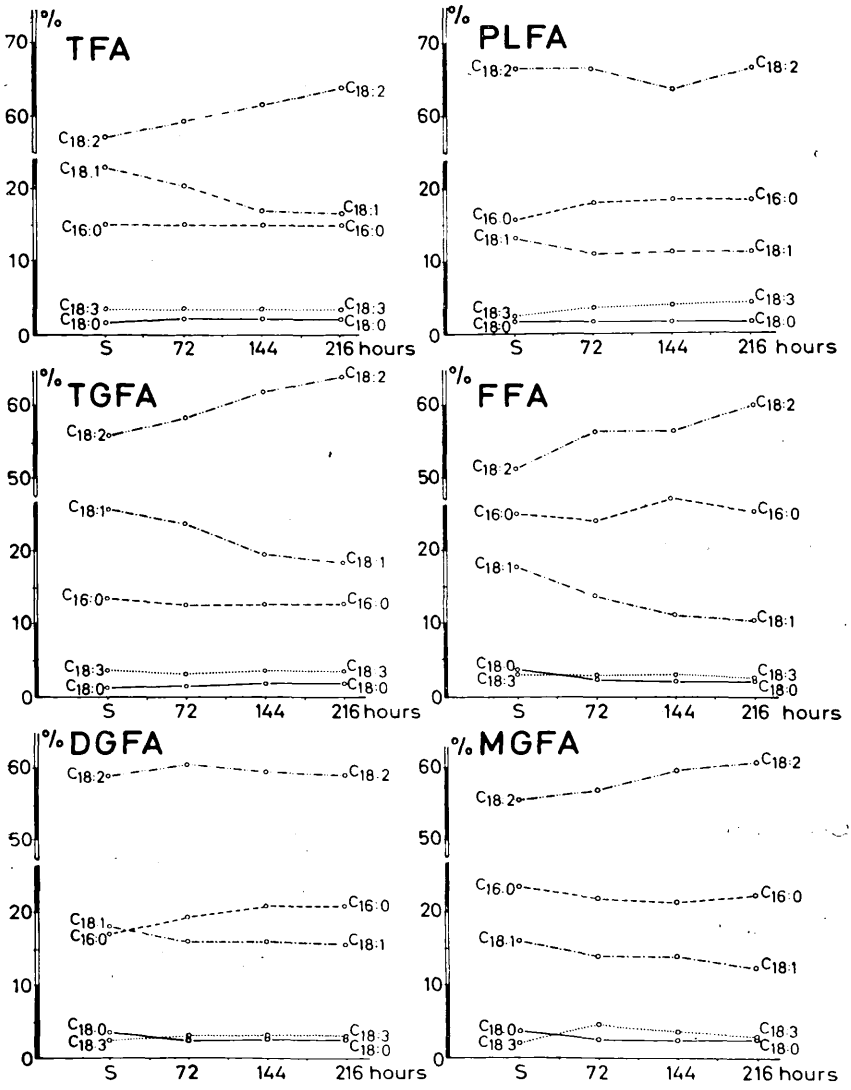

Figure 2.—Changes of fatty acids pattern in lipid classes of degermed seed during germination. Palmitic acid (C16:0), Stearic acid (C18:0), Oleic acid (C18:1); linoleic acid (C18:2), and Linolenic acid (C18:3). Other obreviations as in figure 1.

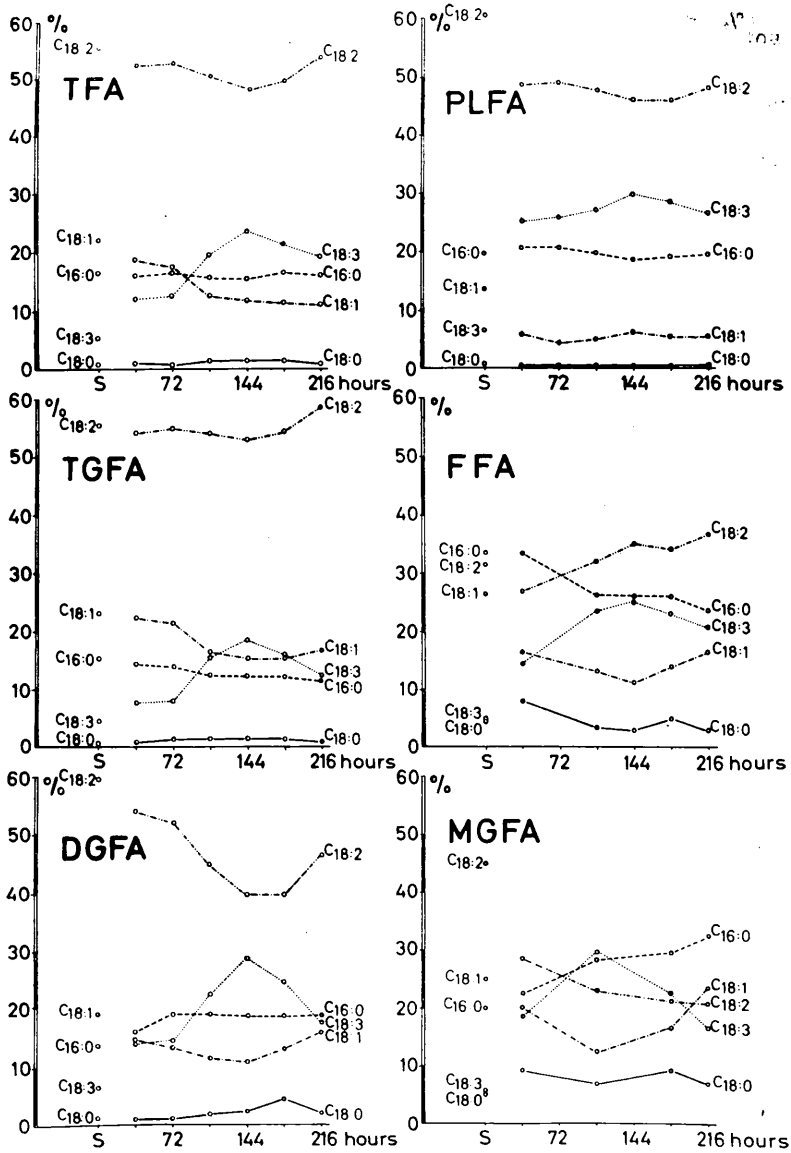

Figure 3.—Changes of fatty acids patterns in lipid classes of germ minus rootlets. Rootlets of steeped sample were not separated. Data for this sample is presented as isolated points. Abreviations as in previous figures.

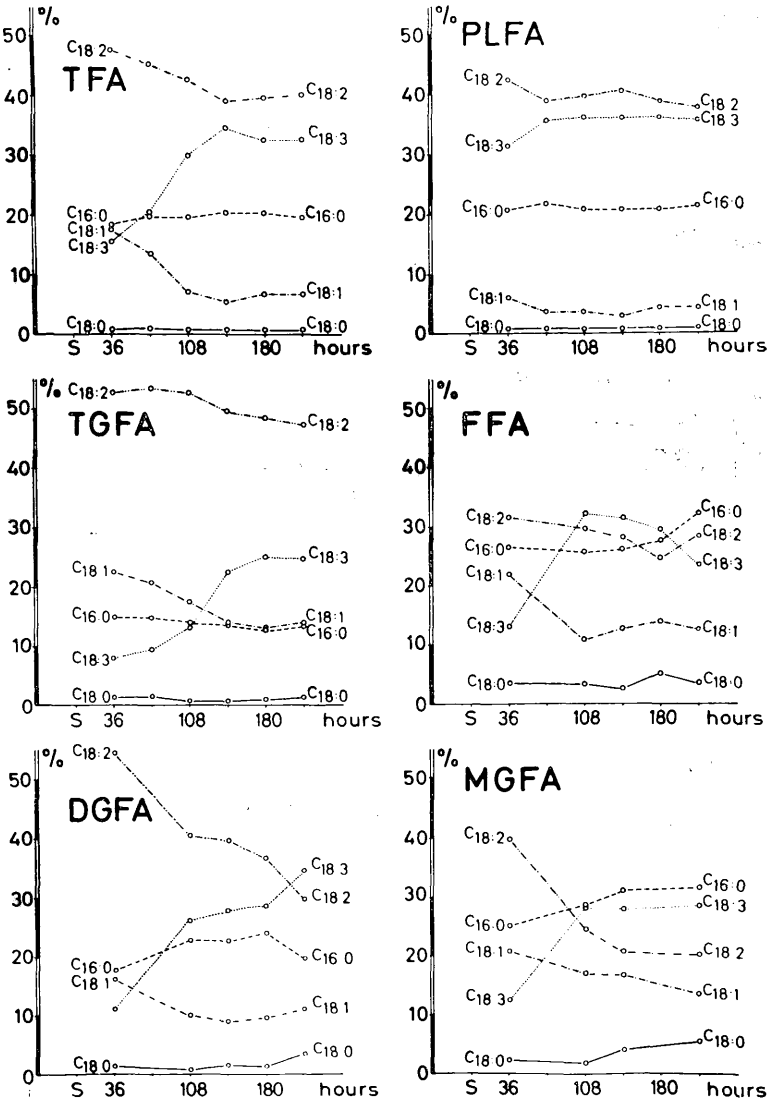

Figure 4.—Changes of fatty acids patterns in lipid classes of rootlets. Abreviations as in previous figures.

wer then faster than that of the other fatty acids in the germination of soy bean. Although Mattson and Volpenheim (15) have reported that linoleic acid is mainly in the beta position in wheat triglycerides, this can hardly explain its accumulation during germination since no specificity for the alfa position has been shown in plant lipases.

The TFA pattern of both the rootlets and the rest of the germ (plumule plus scutellum) undergoes a drastic change during the first half of the germination period (fig. 3 and 4), the proportion of linolenic acid (C 18:3) increasing almost twofold and that of oleic (C 18:1) decreasing. Again the triglycerides can be made responsible for most of the change since the PLFA pattern suffers little alteration. The data in figures 1, 3 and 4 suggests that the original triglycerides present in the germ are used very rapidly in the first stages of germination while new triglycerides with a different fatty acid patern are synthesized in the growing tissues. In the second half of the germination period, when the synthetic process becomes dominant over degradation, both the TFA content of the germ (% of dry matter) and their pattern undergo little change. The FFA, DGFA and MGFA patterns seem to reflect the overlapping of the two processes, changing continuously as it is to be expected from their high turnover.

Conclusions.

Except for the rootlets, where some decrease in their PLFA level is observed, the evolution of this fraction follows rather closely that of non-fat dry matter. The PLFA pattern is different for each part studied but remains constant throughout.

The TGFA are mainly responsible for the changes in lipid content (% of dry matter) and fatty acid pattern observed in the different tissues.

In the endosperm, a preferential degradation of TGFA accounts for the faster utilization of fat over non-fat dry matter. Linoleic acid (C 18:2) depletion is slower and that of oleic acid (C 18:1) faster than the rest of the fatty acids.

In the germ, a very rapid degradation of the existing TGFA takes place at the beginning of germination to be followed by synthesis of new triglycerides with a different fatty acid pattern.

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